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(54) Title: POLYHYDROXYALKANOATE PRODUCTION FROM POLYOLS

(57) Abstract

Organisms are provided which express enzymes such as glycerol dehydratase, diol dehydratase, acyl–CoA transferase, acyl–CoA synthetase β–ketothiolase, acetoacetyl–CoA reductase, PHA synthase, glycerol–3–phosphate dehydrogenase and glycerol–3–phosphatase, which are useful for the production of PHAs. In some cases one or more of these genes are native to the host organism and the remainder are provided from transgenes. These organisms produce poly(3–hydroxyalkanoate) homopolymers or co–polymers incorporating 3–hydroxypropionate or 3–hydroxyvalerate monomers wherein the 3–hydroxypropionate and 3–hydroxyvalreate units are derived from the enzyme catalysed conversion of diols. Suitable diols that can be used include 1,2–propanediol, 1,3–propanediol and glycerol. Biochemical pathways for obtaining the glycerol from normal cellular metabolites are also described. The PHA polymers are readily recovered and industrially useful as polymers or as starting materials for a range of chemical intermediates including 1,3–propanediol, 3–hydroxypropionaldehyde, acrylics, malonic acid, esters and amines.

to the Embden-Meyerof pathway are the phosphofructokinase and fructose 1,6 bisphosphate aldolase.

The term "Entner-Douderoff pathway" refers to a series of biochemical reactions for conversion of hexoses such as as glucose or fructose to the important 3 carbon cellular intermediates pyruvate and glyceraldehyde 3 phosphate without any net production of biochemically useful energy. The key enzymes unique to the Entner-Douderoff pathway are the 6 phosphogluconate dehydratase and a ketodeoxyphosphogluconate aldolase.

The term "high growth methanotrophic bacterial strain" refers to a bacterium capable of growth with methane or methanol as the sole carbon and energy source and which possess a functional Embden-Meyerof carbon flux pathway resulting in a high rate of growth and yield of cell mass per gram of C1 substrate metabolized. The specific "high growth methanotrophic bacterial strain" described herein is referred to as "*Methylomonas* 16a" or "16a", which terms are used interchangeably.

The term "methanotroph" or "methanotrophic bacteria" will refer to a prokaryotic microorganism capable of utilizing methane as its primary carbon and energy source.

As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the protein encoded by the DNA sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by antisense or co-suppression technology. "Substantially similar" also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotide bases that do not substantially affect the functional properties of the resulting transcript. It is therefore understood that the invention encompasses more than the specific exemplary sequences.

For example, it is well known in the art that alterations in a gene which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded protein are common. For the purposes of the present invention substitutions are defined as exchanges within one of the following five groups: